PCT/EP2003/009694

Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RD22220PC IB/mo	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP2003/009694	International filing date (*day/month/year*) 01 September 2003 (01.09.2003)	Priority date (*day/month/year*) 30 August 2002 (30.08.2002)
International Patent Classification (IPC) or national classification and IPC C07K 1/00		
Applicant F. HOFFMANN-LA ROCHE AG		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 March 2004 (05.03.2004)	07 January 2005 (07.01.2005)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP2003/009694

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☒ the description:

pages 1-20, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 1-27, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:

pages 1/1, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

Reference is made to the following documents:

D1: SUMMERS CATHERINE A ET AL: 'Protein renaturalization by the liquid organic salt ethylammonium nitrate' PROTEIN SCIENCE, Vol. 9, No. 10, October 2000 (2000-10), pages 2001-2008, XP009022169 ISSN: 0961-8368

D2: ARMSTRONG D W ET AL: 'Ionic liquids as matrixes for matrix-assisted laser desorption/ionization mass spectrometry.' ANALYTICAL CHEMISTRY, UNITED STATES, 1 AUG 2001, Vol. 73, No. 15, 1 August 2001 (2001-08-01), pages 3679-3686, XP001156233 ISSN: 0003-2700

D3: KULLMANN W: 'PROTEASES AS CATALYSTS FOR ENZYMIC SYNTHESIS OF OPIOID PEPTIDES' JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 255, No. 17, 1980, pages 8234-8238 XP002263435 ISSN: 0021-9258

D4: WO 02 26772 A (HOFFMANN LA ROCHE; JAKUBKE HANS DIETER (DE); BORDUSA FRANK (DE)) 4 April 2002 (2002-04-04)

D5: PARK S ET AL: "Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations." THE JOURNAL OF ORGANIC CHEMISTRY, UNITED STATES, 14 DEC 2001, Vol. 66, No. 25, 14 December 2001 (2001-12-14), pages 8395-8401, XP002263436 ISSN: 0022-3263

D6: EP-A-1 201 657 (CENTRE NAT RECH SCIENT) 2 May 2002 (2002-05-02)

D7: WO 02 26701 A (ABBOTT ANDREW PETER; CAPPER GLEN (GB); SCIONIX LTD (GB); DAVIES DA) 4 April 2002 (2002-04-04)

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

D8: VAN RANTWIJK F ET AL: 'Biocatalytic transformations in ionic liquids' TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, Vol. 21, No. 3, March 2003 (2003-03), pages 131-138, XP004412613 ISSN: 0167-7799

D9: PARK S ET AL. 'Biocatalysts in ionic liquids - Advantages beyond green technology.' CURRENT OPINION IN BIOTECHNOLOGY (2003), 14/4, pages 432-437, XP002263437

D10: EP-A-1 348 767 (HITACHI SOFTWARE ENG) 1 October 2003 (2003-10-01)

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-25, 27	YES
	Claims	26	NO
Inventive step (IS)	Claims	1-25, 27	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-27	YES
	Claims		NO

2. Citations and explanations

D1: The use of the ionic liquid (fused salt) EAN (ethylammonium nitrate) as a refolding additive is advantageous in the one-step renaturation process for increasing the yield in a method to recover denatured-reduced hen egg white lysosome. The results show that EAN is capable of countering the aggregation of the denatured protein.

D2: Ionic liquids (fused salts) were used to coat matrices for UV-MALDI experiments and were tested with various proteins and peptides, particularly bradykinin. Most of the ionic liquids tested showed *inter alia* excellent solvent properties with high vacuum stability.

D3: Proteases are suitable for use as catalysts for the enzymatic syntheses of opioid peptides. Papaine and chymotrypsin were used in examples.

D4: Method for the biocatalytic modification of peptides and proteins using trypsin, chymotrypsin, V8 protease, etc.

D5: The lipase was not inactivated by the influence of ionic fluids: 3-alkyl-1-methylimidazolium tetrafluoroborate shows polarities that are similar to polar organic solvents. The lipase-catalyzed acetylation of 1-phenyl ethanol was just as fast/

enantioselective in ionic liquids as toluene. The lipase-catalyzed (lipase B of *Candida antarctica* (CAL-B)) acetylation of glucose was more regioselective than in ionic liquids.

D6: Imidazolium salts can be used as a solvent in organic processes, particularly in catalytic reactions such as the two-phase conversion of olefins. There is no information regarding protein/peptide-similar synthesis methods.

D7: Ionic liquid systems with examples of fused salts and interesting properties: the use as highly polar solvents in preparative chemistry and as catalysts. One example mentions that two carboxy groups can be present, and the possibility that both COOH groups and NH2 groups can be present is not ruled out, although this is not explicitly preferred.

D8: Various enzymes are catalytically active in ionic liquids/aqueous ionic biphase liquid systems. In particular, lipases retain their activity in a moisture-free environment; the enantioselectivity and the stability are often better than in traditional media. Recommended for the use of biotransformations in amino and nucleic acids.

D9: Enzymes are not inactivated in ionic liquids, as is the case in organic solvents. Moreover, they demonstrate increased stability, particular importance for proteins, peptides as starting materials in preparing pharmaceuticals, etc.

D10: DNA in ionic solvents: protein produced by complementary bonding to a second DNA, which has a marker bound to a first DNA in an ionic liquid, said first DNA being immobilized on a substrate (DNA chip).

1. Claim 26 is not novel with respect to documents D1 and D2. In regard to D1 and D2, it is noted in particular that claim 26, as it is currently worded, does not describe

   a use of ionic liquids in the enzymatically catalyzed peptide bonding between two defined peptides,

   but rather refers in a much more general form to

   the synthesis and/or N-terminal modification of peptides, peptide mimetics and/or proteins.

2. The novelty of claims 1-25 and 27 with respect to documents D1 to D7 is acknowledged.

3. The priority documents show that all of the claims can be granted the right to priority. Therefore, documents D8 and D9 are no longer relevant.
   Document D10 could possibly play a role in a subsequent European procedure (EPC Article 54(3)(4)).

4. For the evaluation of inventive step, it should be assumed from the viewpoint of the problem of interest
   - that of providing a further alternative method for synthesizing peptides and proteins, particularly with regard to a use of ionic liquids in the enzymatically catalyzed peptide bonding between two defined peptides/proteins, wherein it is possible to mix ionic liquids with the conventional solvents or to replace said conventional solvents with ionic liquids in order to suppress hydrolytic and proteolytic secondary reactions -

   that although the use of ionic liquids as a solvent was already known in a method for enzymatically acetylating glucose (not peptides/proteins) according to document D5, which can be considered the closest prior art, as

well as advantageous properties with respect to the activity of the proteins, it **does not** have the desired regioselectivity of reaction between two peptide/protein components: in fact, an unpredictable increase in regioselectivity can be observed that leads to an increased yield (cf. in particular examples 1-4). Therefore, claims 1-15 and 27 involve an inventive step as required by PCT Article 33(3).

5. Contrary to PCT Rule 5.1(a)(ii), the description does not cite documents D1 to D5 or indicate the relevant prior art disclosed therein.